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Morelli, Lidia; Jendresen, Christian Bille; Burger, Robert; Rindzevicius, Tomas; Nielsen, Alex Toftgaard; Boisen, Anja

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SURFACE ENHANCED RAMAN SPECTROSCOPY DETECTION OF p-COUMARIC ACID FROM CELL SUPERNATANT USING GOLD-CAPPED SILICON NANOPILLAR SUBSTRATES

L. Morelli[1], C. Bille Jendresen[2], K. Zor[1], T. Rindzevicius[1], M. Stenbæk Schmidt[1], A. Toftgaard Nielsen[2], A. Boisen[1]

[1] Department of Micro- and Nanotechnology, Technical University of Denmark, Kgs. Lyngby, DENMARK

[2] The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hørsholm, DENMARK

Aim of the Project

The purpose of the project is to use Surface Enhanced Raman Spectroscopy (SERS) to discriminate between two different bacterial populations, based on their p-coumaric acid (pHCA) production. The pHCA concentration is measured in a droplet of diluted supernatant dried on SERS substrates, using a Raman microscope. By analyzing the SERS signal of pHCA from the supernatant, considering the peak height at the characteristic frequency (1169 cm^{-1}) **it is possible to distinguish between a producing and control strain**, as also confirmed by HPLC analysis.

SERS: fabrication and working principle

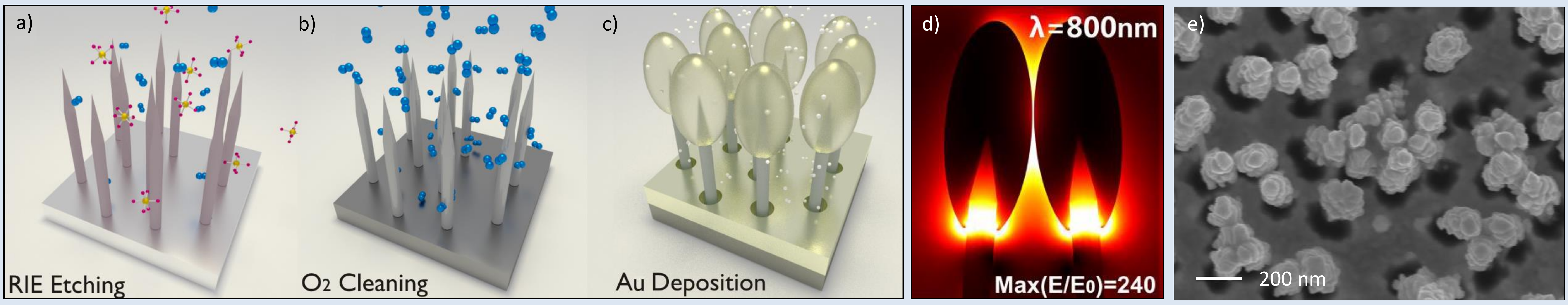

Fig.1: Process steps for Au capped nanopillars (NPs) fabrication (courtesy of Kaiyu Wu) [1]: **(a)** silicon NPs are fabricated by maskless reactive ion etching (RIE); **(b)** O_2 cleaning removes the Si RIE contaminants from the Si surface; **(c)** a Au metal film is deposited by e-beam evaporation. **(d)** When a droplet of solution is dried on the substrate, the surface tension tends to pull the Au NPs together, forming irreversible clusters and trapping the analyte molecules between the NPs. Furthermore, E-field hotspots are created when two NPs lean close to each other. **(e)** SEM picture of leaning pillars.

Bacterial cultures and measurements

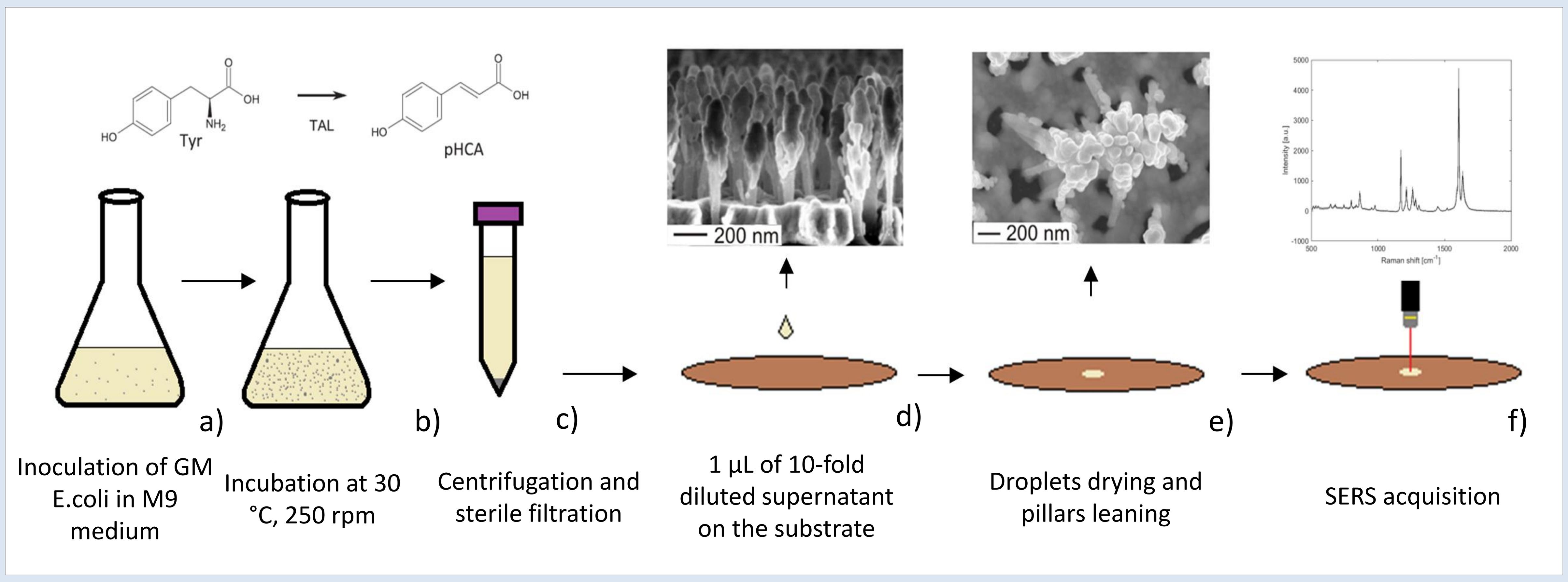

Fig.2: Bacterial culture, supernatant extraction and SERS evaluation: **(a)** and **(b)** genetically modified E.coli are cultured in triplicates [2]; **(c)** aliquots of bacterial solution are extracted at 0, 3, 24 and 48 h, centrifuged and sterile filtered to extract cell supernatant; **(d)** and **(e)** 1 µL of supernatant diluted 10-fold with water is dried on SERS substrates. The SEM pictures show that the pillars stand vertically before wetting, and they lean towards each other after drying. **(f)** 5x5 maps are acquired on the droplet area with a DXR Raman microscope (Thermo Fisher Scientific Inc.) at 780 nm, 1 mW, 10x objective, 25 µm slit.

Salt dilution

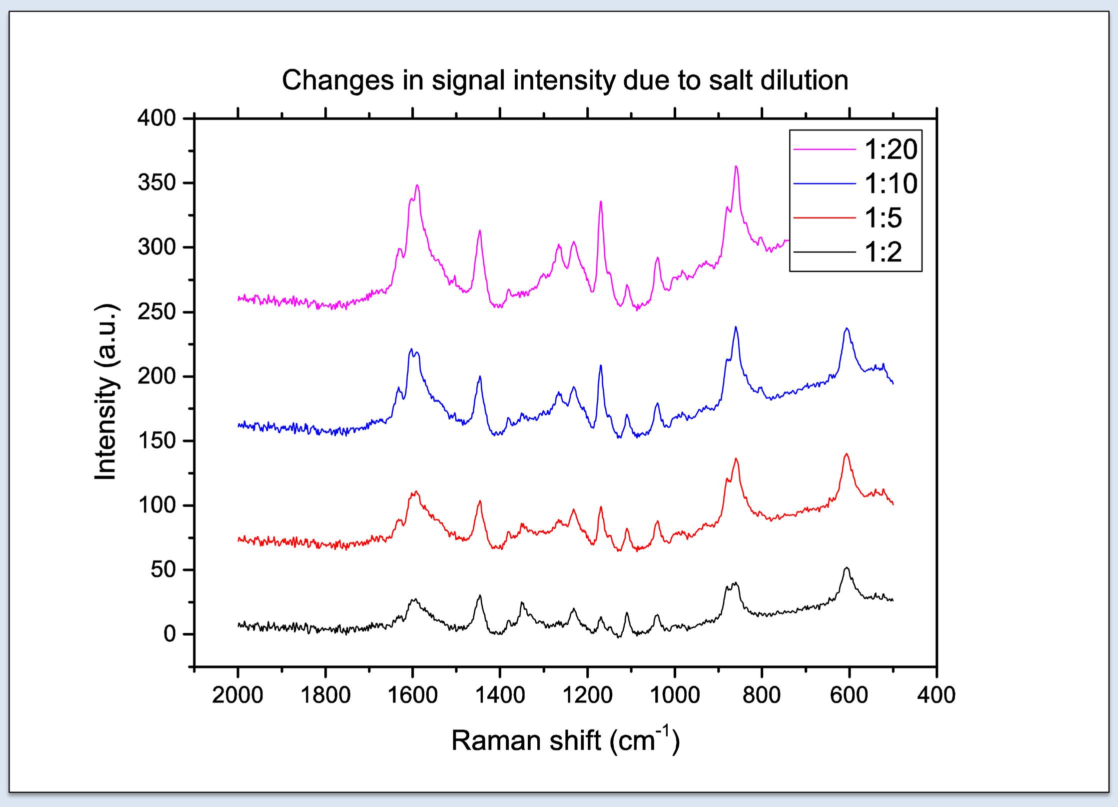

Fig.3: 100 µM pHCA spiked in culture medium diluted with MilliQ water in different ratios (1:2, 1:5, 1:10, 1:20). The signal increases with medium dilution, as higher salt concentration clogs up the active surface and decreases the signal. 1:10 dilution was chosen as a compromise between signal intensity and dilution for measurements in the supernatant.

Validation with HPLC

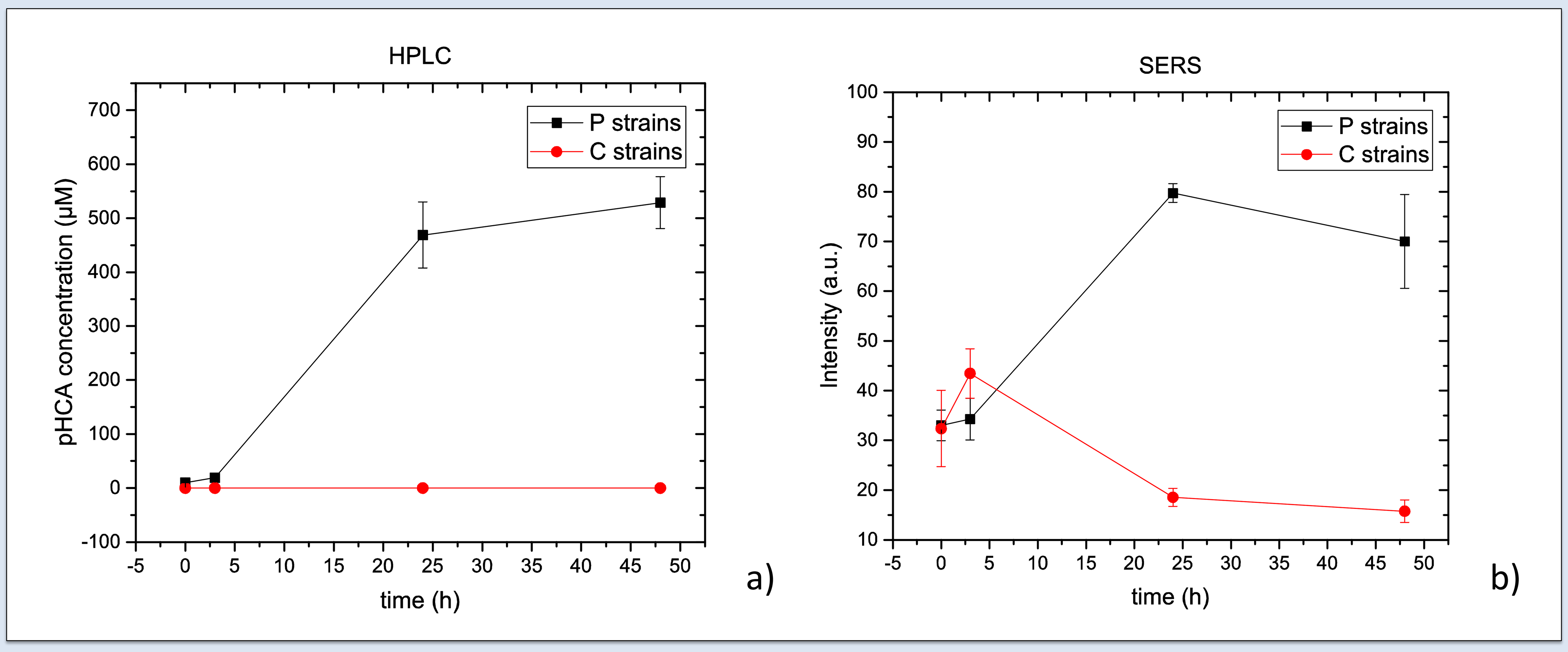

Fig.4: **(a)** Concentration of pHCA in cell supernatant for producing (P, black) and control (C, red) strains measured with HPLC. Each point is the average of 3 measurements, each one obtained from one strain. **(b)** SERS signal at 1169 cm^{-1} after baseline correction. Each point in the graph is the average of 3 maps of 25 points, whereas the error bars represent the standard error of the mean, calculated on the 3 average values.

Outlook and conclusions

In this work we demonstrated that SERS is a rapid and **effective tool for qualitative screening** of bacterial strains, based on the amount of synthetized secondary metabolites (e.g. pHCA). These results open up new **possibilities for high-throughput quantitative analysis.** Currently we are focusing on improving sensitivity by extracting pHCA in organic solvent and on integration of the assays on automated and high-throughput microfluidic platforms, such as lab-on-a-discs.

References

[1] K. Wu, T. Rindzevicius, M. S. Schmidt, K. B. Mogensen, A. Hakonen, and A. Boisen, *J. Phys. Chem. C*, vol. 119, no. 4, pp. 2053–2062, 2015.

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Lidia Morelli
Technical University of Denmark, Department of Micro and Nanotechnology
phone: **+45 91 73 43 40**,
email: **lmor@nanotech.dtu.dk**

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